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GENOME ANNOUNCEMENT

Genome Sequence of the Moderately Halotolerant, Arsenite-Oxidizing Bacterium *Pseudomonas stutzeri* TS44

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We present the draft genome sequence of *Pseudomonas stutzeri* TS44, a moderately halotolerant, arsenite-oxidizing bacterium isolated from arsenic-contaminated soil. The genome contains genes for arsenite oxidation, arsenic resistance, and ectoine/hydroxyectoine biosynthesis. The genome information will be useful for exploring adaptation of *P. stutzeri* TS44 to an arsenic-contaminated environment.

Pseudomonas stutzeri is a Gram-negative, rod-shaped, motile, and nonfluorescent denitrifying bacterium that exhibits metabolic diversity and is widely distributed in the environment (8). Currently, five genome sequences for *P. stutzeri* members have been published, including two nitrogen-fixing bacteria (*P. stutzeri* A1501, CP000304 [19] and *P. stutzeri* DSM4166, CP002622 [20]), a typical lactate utilization bacterium (*P. stutzeri* SDM-LAC, AGSX00000000 [6]), a type strain (*P. stutzeri* CGMCC 1.1803, CP002881 [4]), and a model organism for denitrification (*P. stutzeri* CCUG 16156, AGSL00000000 [15]). *P. stutzeri* TS44 was isolated from a highly arsenic-contaminated soil of a metal (gold, copper, and iron) mine in Huangshi, China (2). This strain is highly resistant to arsenite (with a MIC of 23 mM in chemically defined medium) and displays an arsenite-oxidizing rate of 59.1 $\mu M\ h^{-1}$ (3). It grows vigorously in Luria-Bertani medium with a salt concentration of up to 1.2 M, indicating that strain TS44 is a moderately halotolerant bacterium.

The whole-genome sequence of strain TS44, sequenced by using a Roche 454 GS-FLX apparatus (12) and assembled using the Roche Newbler assembler, includes 4,278,818 bp distributed in 78 contigs, with a depth of 27-fold coverage and an average GC content of 64.4%. In addition, the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html) was used for annotation. The automatic outputs were modified manually based on BLASTP/rps-BLAST and COG analyses (1, 18). The genome is predicted to contain 4,000 putative protein-coding sequences (CDSs), 55 tRNA genes, and 5 rRNA genes. The Mauve software program was employed for further genome comparison and orthology analysis (5). Multiple-genome comparisons indicated strain TS44 was most related to strain CGMCC 1.1803 (64.37%), followed by strain DSM4166 (63.1%), strain SDM-LAC (62.5%), strain ATCC 14405 (62.1%), and strain A1501 (62.0%), based on ortholog CDS analysis (coverage, ≥50%; identity, ≥30%). The six genomes shared 2,251 CDSs, which may comprise the core genome of *P. stutzeri*, and 978 CDSs (of 4,000 [24.5%]) were only found in strain TS44.

The genome contains at least 20 genes encoding functions related to arsenite oxidation and resistance, mainly located on contig 00030 (AJXE01000030; *ars* operon, *arsC1-arsR-arsC2*-ACR3-*arsH*-DSP-GAPDH-MFS, and an adjacent *ars-aox* operon, *arsD-arsA-aioA-aioB*) and contig 00031 (AJXE01000031; *ars* operon, ACR3-*arsR-arsH*-GAPDH-MFS) (9, 11, 13). Notably, *aioAB* (encoding arsenite oxidase) was absent in the other five *P. stutzeri* genomes, suggesting recent acquisition of *aioAB* by strain TS44 via horizontal gene transfer. However, a two-component system, *aioS aioR*, involved in regulating the expression of *aioAB*, was not identified in the genome, indicating a potentially novel regulatory mechanism (10, 14). In addition, numerous genes responsible for resistance to other metals (copper, mercury, chromate, cadmium, and zinc) were also identified.

Genomic comparison demonstrated that all six sequenced *P. stutzeri* strains possess a complete cluster of ectoine/hydroxyectoine biosynthetic genes (*ectABCD-ask*) (16, 17). However, this entire *ectABCD-ask* cluster was not identified in the genomes of other *Pseudomonas* species (7). Compared to other *Pseudomonas* species, ectoine/hydroxyectoine biosynthesis is presumably a common strategy for *P. stutzeri* to survive under high-osmolarity conditions.

Nucleotide sequence accession numbers. The results of this genome shotgun project have been deposited with DDBJ/EMBL/GenBank under the accession number AJXE00000000. The version described in this paper is the first version, AJXE01000000.

ACKNOWLEDGMENTS

This work was supported by grants from the National Natural Science Foundation of China (no. 30970075) and a Major International Collaborative Project of National Natural Science Foundation of China (no. 31010103903).

Sequencing was performed at the University of Arizona Genetics Core.

REFERENCES

1. **Altschul SF, et al.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25**:3389–3402.

Received 24 May 2012 Accepted 5 June 2012

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doi:10.1128/JB.00907-12

2. **Cai L, Liu G, Rensing C, Wang G.** 2009. Genes involved in arsenic transformation and resistance associated with different levels of arsenic-contaminated soils. BMC Microbiol. **9**:4. doi:10.1186.1471-2180-9-4.
3. **Cai L, Rensing C, Li X, Wang G.** 2009. Novel gene clusters involved in arsenite oxidation and resistance in two arsenite oxidizers: *Achromobacter* sp. SY8 and *Pseudomonas* sp. TS44. Appl. Microbiol. Biotechnol. **83**:715–725.
4. **Chen M, et al.** 2011. Complete genome sequence of the type strain *Pseudomonas stutzeri* CGMCC 1.1803. J. Bacteriol. **193**:6095.
5. **Darling AC, Mau B, Blattner FR, Perna NT.** 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res. **14**:1394–1403.
6. **Jiang T, et al.** 2012. Genome sequence of *Pseudomonas stutzeri* SDM-LAC, a typical strain for studying the molecular mechanism of lactate utilization. J. Bacteriol. **194**:894–895.
7. **Kurz M, Burch AY, Seip B, Lindow SE, Gross H.** 2010. Genome-driven investigation of compatible solute biosynthesis pathways of *Pseudomonas syringae* pv. *syringae* and their contribution to water stress tolerance. Appl. Environ. Microbiol. **76**:5452–5462.
8. **Lalucat J, Bennasar A, Bosch R, GarcíA-Valdés E, Palleroni NJ.** 2006. Biology of *Pseudomonas stutzeri.* Microbiol. Mol. Biol. Rev. **70**:510–547.
9. **Lebrun E, et al.** 2003. Arsenite oxidase, an ancient bioenergetic enzyme. Mol. Biol. Evol. **20**:686–693.
10. **Li X, et al.** 2012. Genome sequence of the highly efficient arsenite-oxidizing bacterium Achromobacter arsenitoxydans SY8. J. Bacteriol. **194**:1243–1244.
11. **Lin Y, et al.** 2012. Draft genome sequence of *Halomonas* sp. strain HAL1, a moderately halophilic arsenite-oxidizing bacterium isolated from gold-mine soil. J. Bacteriol. **194:1** 199–200.
12. **Margulies M, et al.** 2005. Genome sequencing in microfabricated high-density picolitre reactors. Nature **437**:376–380.
13. **Mukhopadhyay R, Rosen BP, Phung LT, Silver S.** 2002. Microbial arsenic: from geocycles to genes and enzymes. FEMS Microbiol. Rev. **26**: 311–325.
14. **Muller D, et al.** 2007. A tale of two oxidation states: bacterial colonization of arsenic-rich environments. PLoS Genet. **3**:e53. doi:10.1371/journal/pgen.0030053.
15. **Peñaa A, et al.** 2012. Draft genome of *Pseudomonas stutzeri* strain ZoBell (CCUG 16156), a marine isolate and model organism for denitrification studies. J. Bacteriol. **194**:1277–1278.
16. **Seip B, Galinski EA, Kurz M.** 2011. Natural and engineered hydroxyectoine production based on *Pseudomonas stutzeri ectABCD/ask* gene cluster. Appl. Environ. Microbiol. **77**:1368–1374.
17. **Stöveken1 N, et al.** 2011. A specialized aspartokinase enhances the biosynthesis of the osmoprotectants ectoine and hydroxyectoine in *Pseudomonas stutzeri* A1501. J. Bacteriol. **193**:4456–4468.
18. **Tatusov RL, Galperin MY, Natale DA, Koonin EV.** 2000. The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Res. **28**:33–36.
19. **Yan Y, et al.** 2008. Nitrogen fixation island and rhizosphere competence traits in the genome of root-associated *Pseudomonas stutzeri* A1501. Proc. Natl. Acad. Sci. U. S. A. **105**:7564–7569.
20. **Yu H, et al.** 2011. Complete genome sequence of the nitrogen-fixing and rhizosphere-associated bacterium *Pseudomonas stutzeri* strain DSM4166. J. Bacteriol. **193**:3422–3423.